CLAIMS:

1. An immunobead-flow cytometry assay for simultaneously detecting a plurality of antigens or antibodies in a sample comprising the steps of:

coating a plurality of distinct latex beads each with at least one of a unique antigen or antibody,

incubating the coated beads with serum,

labeling the bead/serum mixture with anti-human fluorescently labeled antibodies,

analyzing the labeled beads on a flow cytometer with at lease one of a blank and isotopic control as the negative standard, and forward scatter versus fluorescence used to detect positivity.

2. An assay as claimed in claim 1 further comprising the steps of:

binding one of highly purified Scl-70, RNP, SM, SS-A, SS-B and dsDNA antigens to one of 3, 4, 5, 6, 7 and 8 μm latex beads,

placing diluted patient serum into test tubes containing a mixture of the six antigen coated beads,

incubating the serum and beads so that any antibody for a specific antigen will bind to that specific bead,

incubating the beads with goat-anti-human IgG conjugated with a fluorochrome such as fluorescein isothiocyanate (FITC), to allow the conjugate to bind immunologically to the anti-antigen IgG of the antigen-antibody complex, forming a "sandwich" consisting of bead - antigen - 1° antibody - 2° antibody - FITC,

analyzing the sandwich using a flow cytometer having a laser excitation wavelength of about 488 nm and producing emission wavelengths detected by photomultipliers, and

converting the fluorescent analog signals into at least two parameter histogram expressing forward light scatter (Y-axis) versus fluorescence intensity (X-axis) and analyzed quantitate or semi-quantitate on linear fluorescent (X-axis) only.

3. An assay as claimed in claim 1 further comprising:

a. determining which antigen coating buffer of carbonate buffer and phosphate buffered

saline yields highest binding capacity to latex beads,

b. establishing titers of both antibodies against the coated beads and running several experiments to maximize signals obtained at different antigen concentrations (mean channel fluorescence),

c. incubating the antigen/serum mixture for several minutes and washing with either carbonate buffer or phosphate buffered saline,

d. washing antigen coated beads in a buffer of at least one of phosphate buffered saline, 0.3% protein in phosphate buffered saline, or carbonate buffer,

e. determining the background of unlabelled beads,

f. if background exists, decreasing to near baseline values,

g. finding proper dilution of patient and control sera and adding to coated beads,

h. incubating for optimal time and washing with buffer of phosphate buffered saline and carbonate buffer.

i. determining the optimum amount of labeled goat-anti-human $f(ab')^2$ antibody by titration and using as the indicator system,

j. repeating step h,

k. adding 1 mL of buffer of phosphate buffered saline and carbonate buffer, and

l. reading on flow cytometer.

4. An assay as claimed in claim 1 comprising the steps of

a) coating each of a plurality of discrete beads with a particular antigen or antibody using a buffer,

b) incubating bead/serum mixture for several minutes and washing with at least one of carbonate buffer and phosphate buffered saline,

c) washing incubated beads in buffer,

d) determining the background of unlabelled beads,

e) if background exists, decreasing to near baseline values,

f) finding proper dilution of patient and control sera and adding to coated beads,

g) incubating for optimal time,

h) determining the optimum amount of a labeled anti-human antibody by titration and using as the

indicator system,

i) repeating step g), and

j) reading on flow cytometer.

5. An assay as claimed in claim 1 using an anti-viral screening kit for simultaneous detection of anti-antibodies to antigens selected from one or more of the following: HTLV, HCV, EBV, HIV, CMV HbsAg, Hbc in serum, Hepatitis Surface antigen, core antigen, HIVI/I, HTL VI/II, and Hepatitis C antigen, as an aid in the diagnosis of viral infection, the kit comprising the steps of:

a) allowing test components and patient samples to warm to room temperature before use, returning promptly to refrigerator after use,

b) properly labeling sufficient numbers of test tubes to identify positive and negative controls and patient samples,

c) adding a specific amount of bead solution into each of the labeled test tubes,

d) preparing dilutions of the Positive and Negative Controls, and the patient samples,

e) mixing sample dilutions gently by withdrawing and expelling in a pipette or vortexing,

f) transferring an amount of each diluted control or patient sample into corresponding test tube,

g) gently vortexing and incubating at room temperature (20-30°C) for 15 to 30 minutes,

h) adding one drop (50 μL) of fluorescenated conjugate to each tube,

i) gently vortexing and incubating for 15 to 30 minutes at room temperature in the dark, and

j) analyzing on flow cytometer.

6. A fluorescent immuno-bead assay kit for use in conjunction with flow-cytometry for the simultaneous detection of one or more of the antinuclear antibodies to RNP (ribonucleoprotein) seen in mixed connective disease, systemic lupus erythematosis (SLE), Sjogren's syndrome, scleroderma and polymyositis; Sm (Smith antigen) in SLE; SS-A in Sjogren's syndrome and SLE; SS-B in Sjogren's syndrome and SLE; dsDNA in SLE; and Scl-70 in scleroderma, these

antibodies being commonly encountered in the so-called rheumatic diseases, the kit comprising:

(a) at least one particle sized latex bead having sizes selected from 3 μm, 4 μm, 5 μm, 6 μm, 7 μm, and 8 μm,

(b) at least two antigens selected from Sm/RNP Complex antigen, Sm antigen, SS-A (Ro) antigen, SS-B (La) antigen, Scl-70 antigen, dsDNA antigen,

(c) at least two anti-antigen selected from Anti-RNP, Anti-Sm, Anti-SS-A (Ro), Anti-Sm, Anti-SSB (La), Anti-Scl-70, anti-dsDNA,

(d) Goat anti-human IgG $F(ab')^2$-FITC,

(e) Sodium Carbonate,

(f) Sodium Bicarbonate, and

(g) Albumin, bovine,

whereby beads, antigen and anti-antigen are selected for use in the kit based on target antigens or antibodies being tested.

7. An assay kit as claimed in claim 6 wherein beads with antigens coated on the surface are impregnated with dye and assayed by size and/or fluorescent properties.

8. An assay as claimed in claim 6 wherein antigens are grouped in predetermined combinations.

9. An assay kit as claimed in claim 6 designed to simultaneously detect several antinuclear antibodies in patient sera utilizing antigen coated microspheres to different sizes, binding of antibody to spheres is detected by FITC labeled anti-human IgG and flow cytometry, with each individual antibody detected because of binding to a different sized sphere which is determined by light scatter, the kit comprising at least one antigen coated bead selected from:

a) 3 μm latex beads coated with Scl-70 antigen,

b) 4 μm latex beads coated with Sm/RNP complex antigen,

c) 5 μm latex beads coated with Sm antigen,

d) 6 μm latex beads coated with SS-A (Ro) antigen,

e) 7 μm latex beads coated with SS-B (La) antigen, and

f) 8 μm latex beads coated with dsDNA antigen.

10. An assay as claimed in claim 6 for anti-SLE screening for the simultaneous detection of anti-antibodies to the antigens RNP, Sm, SS-A (Ro), SS-B (La), dsDNA and Scl-70 in serum as an aid in the diagnosis of certain co-called rheumatic or connective tissue diseases, such as systemic lupus erythematosis (SLE), Sjogren's syndrome, scleroderma, and polymyositis, the assay comprising the steps of:

a) adding 15 μL of sample to 600 μL of RNP, Sm, SS-A (RO), SS-B (La), dsDNA and Scl-70 coated bead solution, and mixing well,

b) incubating at room temperature for 15 to 30 minutes,

c) placing one drop of fluorescenated conjugate into each tube, mixing well,

d) incubating at room temperature, in the dark, for 15 to 30 minutes, and

e) reading of flow cytometer.

11. An assay as claimed in claim 6 for anti-viral screening for the simultaneous detection of anti-antibodies to antigens selected from one or more of: HTLV, HCV, EBV, HIV, CMV HbsAg, Hbc in serum, Hepatitis Surface antigen, core antigen, HIVI/I, HTL VI/II, and Hepatitis C antigen, as an aid in the diagnosis of viral infection, the assay comprising the steps of:

a) adding an appropriate amount of sample to a bead solution including one or more antigens selected from CMV, EBV, HbsAg, Hbc, HTLV, HCV, HIV and mixing well,

b) incubating at room temperature for 15 minutes,

c) placing one drop of fluorescenated conjugate into each tube, mixing well,

d) incubating at room temperature, in the dark, for 15 to 30 minutes, and

e) reading of flow cytometer.

12. A no-wash fluorescent immunobead assay comprising the steps of:

a) allowing reagents to come to room temperature,

b) gently inverting antigen coated bead mixture until an even distribution of bead product is

observed,

c) labeling test tubes for controls and patients,

d) adding multiple bead suspension to each tube,

e) diluting patient and control serum 1:100 in protein buffer,

f) adding 15 µL of diluted serum to appropriate test tubes,

g) gently vortexing and incubating for 15 minutes at room temperature,

h) making a dilution of goat anti-human $F(ab')^2$ IgG FITC in protein buffer,

i) adding 50 µL of diluted conjugate to each tube,

j) gently vortexing and incubating for 15 minutes at room temperature, in the dark, and

k) analyzing on flow cytometer.

13. A no-wash detection method using the assay of claim 1, the method comprising the steps of:

a) coating each of the plurality of discrete beads with a particular probe,

b) gently inverting the probe coated bead mixture until an even distribution of bead product is observed,

c) labeling test tubes for Blank, Controls, and Patients,

d) adding equal quantities of bead suspension to each tube,

e) diluting patient control serum to a specific volume with protein buffer,

f) adding equal quantities of diluted serum to appropriate test tubes,

g) gently vortexing and incubating at room temperature,

h) making a dilution of labeled anti-human antibody in protein buffer,

i) adding equal quantities of diluted conjugate to each tube,

j) gently vortexing and incubating at room temperature, and

k) analyzing on flow cytometer.

14. The method as claimed in claim 13 wherein the probe is selected from at least one of:

a) antigens selected from one or more of RnP, Sm, SS-A, SS-B, Scl-70, dsDNA, Jo-1, centromeres, histones or other antigens related to rheumatic diseases, viruses, bacteria or cellular

material,

b) antibodies selected from one or more of anti-p24, anti-HTLV, OKT3,

c) chemicals selected from one or more of IL-2, toxins, drugs,

d) microorganisms selected from one or more of E.coli, HTLV, viruses, other bacteria

e) cell components selected from one or more of IL-2R, Glycoproteins,

f) DNA - double stranded complement strands,

g) RNA - viral RNA, and

h) cariolipin, pollen, metals, or recombinant proteins.

15. A no-wash assay comprising the steps of:

a) washing beads,

b) coating beads,

c) suspending coated beads,

d) mixing different beads,

e) reacting beads,

f) reacting beads with anti-human FITC labeled antibodies, and

g) reading beads on flow cytometer.